EXHIBIT C2

2d ET Stim. SnMEL28 c̄ Tikhdon
& 1ETD

- Extracts made c̄ RIPA/DC pH8.0, 20mM DTT, Aprotinin
- ET added 7pm, Inhib added 7pm (24hr later) PMSF
- cells harvested @ ~1:30pm Day 2 (~41 hours) → 42.5hrs Vanadate

PM	OD_{595}(2λ)	γ/λ	2.5γ	1γ
2N	.264	1.9	1.32	0.53
2E	.346	2.8	0.89	0.36
2N/1nM	.290	2.2	~~0.46~~ 1.14	0.45
2E/1nM	.326	2.5	1.0	0.4
2N/5nM	.308	2.4	1.04	
2E/5nM	.349	2.8	0.89	
2N/10nM	.261	1.9	1.32	
2E/10nM	.345	2.8	0.89	

Gel ① E CAD 2.5γ

2N 2E 2N/1 2E/1 2N/5 2E/5 2N/10 2E/10

Gel ② βCAT 1.25γ pY20 2.5γ

Ⓜ 2N 2E 2N/5 2E/5 Ⓜ 2N 2E 2N/5 2E/5

Cyt	OD_{595}(4λ)	γ/λ
2N	.374	1.6
2E	.482	2.2

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Addendum:

- InM IETD inhibits GCAD downreg and p120 downreg
- InM IETD suppresses βCAT in both stimulated samples and unstimulated controls

concl:

- InM IETD is sufficient to inhib E-CAD downreg
- higher concentrations may titrate out factors which participate in apoptosis
- difficult to say if βCAT downreg is required for ↓ECAD since IETD suppresses βCAT in unstimulated samples
- ↓ p120 may be a critical factor.

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Results

(1) ECAD

- Gel somewhat overloaded
- ICTD inhibits ECAD downregulation @ [1nM] but not @ 5nM and 10nM concentrations
- ? ↑ mobility of ECAD in 2E/ICTD 1nM sample
- More potent ET-1 induced down regulation c̄ increasing concentrations of inhibitor

Gel (2)

p120
- SHIFT APPRECIATED! in ET-1 stimulated sample
- 5nM ICTD suppress p120 levels @ baseline
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βCAT - 5nM ICTD inhibits βCAT downreg
- 2E sample ran strangely so not sure if shift present

↳ Repeating ECAD

(1) 1γ | 1nM | p120 2.5 | 1nM

M	2N	2E	2N	2E	M	2N	2E	2N	2E
	5.3	3.6	4.5	4		13.3	9	11.3	10

(2) βCAT 1γ | 1nM | ext 006 2.5

M	2N	2E	2N	2E	[illegible]	2N	2E	4E	(M)
	5.3	3.6	4.5	4		7	8.3	7.8	

←